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Huperzine A: Behavioral and Pharmacological Evaluation in Rhesus Monkeys

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14. ABSTRACT
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ABSTRACT

Huperzine A is potentially superior to pyridostigmine bromide as a pretreatment for nerve agent intoxication because it inhibits acetylcholinesterase both peripherally and centrally, unlike pyridostigmine, which acts only peripherally. Using rhesus monkeys, we evaluated the time course of acetylcholinesterase and butyrylcholinesterase inhibition following four different doses of -(-)huperzine A: 5, 10, 20, and 40 ug/kg. Acetylcholinesterase inhibition peaked 30 minutes after intramuscular injection and varied dose dependently, ranging from about 30% to 75%. Subsequently, cognitive-behavioral functioning was also evaluated at each dose of huperzine A using a six-item serial-probe recognition task that assessed attention, motivation, and working memory. The results demonstrate that huperzine A can selectively and reversibly inhibit acetylcholinesterase without cognitive-behavioral side effects, thus warranting further study.

INTRODUCTION

Current nerve agent pretreatment relies on the use of pyridostigmine (PYR) bromide tablets taken every eight hours over several days to achieve a target red blood cell (RBC) inhibition of acetylcholinesterase (AChE) of approximately 20-40% [1-6]. PYR is a reversible carbamate AChE inhibitor that prevents some AChE from binding with the nerve agent, thereby preventing lethality. However, PYR is a polar compound that does not cross the blood-brain barrier and, thus, only inhibits peripheral AChE. Therefore, PYR does not directly protect against nerve agent-induced central nervous system (CNS) injury or centrally mediated seizures and subsequent brain damage.

A centrally acting nerve agent pretreatment will potentially be more effective than PYR. Indeed, physostigmine (a nonpolar tertiary amine that penetrates the CNS) has been demonstrated to afford considerable protection against nerve agents in a variety of species [7-11]. More recently, several laboratories have examined huperzine A (HUP) as a centrally acting pretreatment compound [12-15]. For example, Lallement [13] implanted primates with an osmotic pump containing either PYR or HUP at equipotent doses to produce approximately 20% RBC AChE inhibition prior to challenge with cumulative doses of soman. Monkeys given HUP required 1.55 times more soman before the onset of convulsions and epileptic activity, demonstrating the greater efficacy of HUP against soman intoxication. HUP may be more effective than physostigmine at preventing nerve agent intoxication because it does not significantly inhibit butyrylcholinesterase (BChE), allowing this endogenous scavenger to provide protection, albeit limited, against organophosphorus nerve agents. Supporting this view, Grunwald et al. [12] demonstrated greater protective ratios against soman challenge with huperzine relative to physostigmine.

To be used effectively as a pretreatment, a compound must be devoid of undesirable cognitive-behavioral effects. Although PYR has an excellent safety record in humans, it can produce undesirable side effects such as nausea, gastrointestinal symptoms, abdominal pain, diarrhea, excessive sweating, and frequent urination at current therapeutic levels [2]. Even slight performance decrements could be significant in a battlefield scenario. The concern is even greater when the pretreatment compound acts upon the CNS. The undesirable behavioral effects of physostigmine are well documented [16-21]. In contrast, HUP appears to have an excellent behavioral safety profile in humans and has been evaluated for its ability to relieve memory deficits associated with Alzheimer's disease and vascular dementia [22-23]. Unfortunately, the safety assessment of HUP on healthy adults (not elderly or pharmacologically challenged subjects) has been limited, and carefully controlled studies using accepted, automated, and standardized tests of cognition and performance in primates have been lacking. We endeavored to evaluate the safety of several doses of HUP on the cognitive-behavioral performance of rhesus monkeys using a computerized touchscreen task that has been shown in Department of Defense laboratories to be sensitive to cholinergic challenge. In addition, we characterized the time course of acetylcholinesterase and butyrylcholinesterase inhibition at four different doses of HUP injected intramuscularly that encompassed the therapeutically relevant dose (i.e., a dose that, like pyridostigmine, produces approximately 30% peripheral inhibition of AChE).

METHOD

The experimental protocol was approved by the Animal Care and Use Committee at the Walter Reed Army Institute of Research and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, National Academy Press, 1996, and the Animal Welfare Act of 1966, as amended.

Subjects

Six rhesus monkeys (named A1, A2, A3, A4, A5, and A7) were used to evaluate the time-course of cholinesterase inhibition, two at each huperzine dose. A7 was male and weighed 7.9 kg. The remaining monkeys were female and ranged in weight from 4.4 to 5.5 kg. Only A1, A2, A3, and A4 were used to assess the behavioral effects of huperzine, and this assessment occurred several weeks after the cholinesterase time-course evaluation was completed.

Drug

HUP (obtained from the Division of Biochemistry, WRAIR) was dissolved in sterile saline to a concentration of 800 ug/mL (expressed as the weight of the salt). The volume injected was varied to examine four different doses: 5, 10, 20, and 40 ug/kg.

Cholinesterase Evaluation

Circulating butyrylcholinesterase (BChE) and acetylcholinesterase (AChE) were sampled from the saphenous vein of conscious monkeys restrained in a Primate Products (Immokalee, FL) restraint chair and measured using the WRAIR whole blood cholinesterase assay [24] at the following time points: 0 (pre-injection baseline), 0.5, 1, 1.5, 2, and 24 hours following intramuscular (IM) injection.

Behavioral Apparatus

The subjects were tested unrestrained in their home cages [25]. A 35.6-cm (14-in.) capacitive touch screen monitor (GoldStar StudioWorks, model GLD 45I, Microtouch Systems, Inc., Methuen, MA) was attached to the front wall of each cage, with the center of the screen 38.9 cm above the chamber floor. Because screen touches are difficult to execute around the screen's perimeter, the effective area of the screen was reduced by 1.5 cm on all four sides. Banana-flavored food pellets (750 mg, Bio-Serv Inc., Frenchtown, NJ) were delivered by a pellet dispenser (BRS/LVE Model QNB-400 1) into a food cup (7.9 X 10.8 X 7.6 cm) positioned in the front of the test chamber, accessible through an aperture (7.6 cm wide X 5.4 cm high) centered 15.1 cm below the lower edge of the touch screen and 11.6 cm above the chamber floor. A computer, running a custom-written Visual Basic 6.0 routine, was used to control experimental events and collect all data.

Behavioral Procedure

Each daily session consisted of 240 trials and sessions lasted approximately 1 hour. On each trial, six unique sample stimuli (list items) were presented sequentially, separated by a 1-s interstimulus interval (ISI) during which the screen was blank. Each list item was a compound stimulus comprised of two superimposed, randomly selected

ASCII characters of different size and color. The individual characters ranged from about 0.3 to 2.7 cm in length and 0.3 to 2.7 cm in width. Because the same ASCII character could be selected for a particular sample stimulus, one character was 15% smaller than the other and was offset slightly above and to the left of the other to avoid perfect overlap and to achieve a greater diversity of compound sample stimuli. The RGB color saturation of each ASCII character ranged from 0 to 255. To exclude extremely dark characters but not true colors, at least one of the three saturation levels had to exceed 79. Each list stimulus was displayed in the top-center portion of the screen, about 13.5 cm from the left edge of the screen and about 4 cm from the top of the screen to the center of the stimulus. Each list item was presented for 3 s or until it was touched, at which point it was terminated and the ISI was initiated. After presentation of the sixth sample stimulus, the screen was blank throughout the 1-s probe delay (retention interval) that preceded the choice period. During the 15-s choice period a probe stimulus was displayed in the lower-left or lower-right portion of the screen, and a standard or default stimulus (a 6.6-cm white square) was presented in the other portion of the screen, with equal frequencies of presentation on both sides. The probe item was a compound stimulus that matched a list item on half of all trials (120). Across these "matching" trials, probe items matched list items at each of the six serial positions with equal frequency (20 at each serial position). On matching trials, touching the probe stimulus was considered correct. In contrast, on "non-matching" trials the probe stimulus was not among those listed (novel) and touching the default stimulus was considered correct. A correct choice response immediately produced a conditioned reinforcer (the entire screen turned white for 0.25 s) every time, but produced a food pellet only 33.3% of the time, determined randomly by the computer (this probabilistic reinforcement schedule was used to maintain high, consistent levels of responding and avoid possible satiation). Touching the opposite stimulus was considered incorrect. Choice periods that elapsed without a response ended after 15 s and were considered incorrect. A 4-s intertrial interval (or ITI, during which the screen was blank) separated each trial, regardless of whether a choice was correct or incorrect. A response during the ITI reset the interval, although few such responses occurred. Only one injection was given per week to allow sufficient recovery time between doses. Sessions began exactly 30 min following injection of the test compound or saline (0.3 mL as a vehicle control). The order of doses was 0, 5, 10, 20, 40, and 0 ug/kg IM.

RESULTS

During the time course study, a toxic signs evaluation was conducted for each animal at each time point, and no overt clinical signs of intoxication were observed at any time.

Cholinesterase Results

Figure 1 characterizes the time course of AChE inhibition over a 24-h period for each of four doses (as differentiated in the legend). The time course of inhibition was similar across doses and approximated baseline levels by 24 h postinjection (except at the highest dose). Peak inhibition was observed at 30 minutes for all doses, and the peak inhibition was dose dependent.

Figure 2 shows peak levels of inhibition of AChE and BChE (measured at 30 minutes postinjection). The peak level of AChE inhibition was a function of dose and ranged from 31 to 74%. BChE inhibition ranged from 0 to 10%. This demonstrates the relative selectivity of HUP for AChE over BChE. A linear regression was conducted for BChE as a function of HUP dose, and the fit was very good. R-squared equaled .935, the slope equaled 0.333 (p=.03), and the y-intercept equaled -4.1 (p=.10, NS). For AChE, a hyperbolic model fit the data best. The formula was $y=ax/(b+x)$, where a equals the asymptotic maximum and b equals the value of x producing the half-maximal response. R-squared equaled .993, a equaled 94.79 (p=.002), and b equaled 10.62 (p=.015).
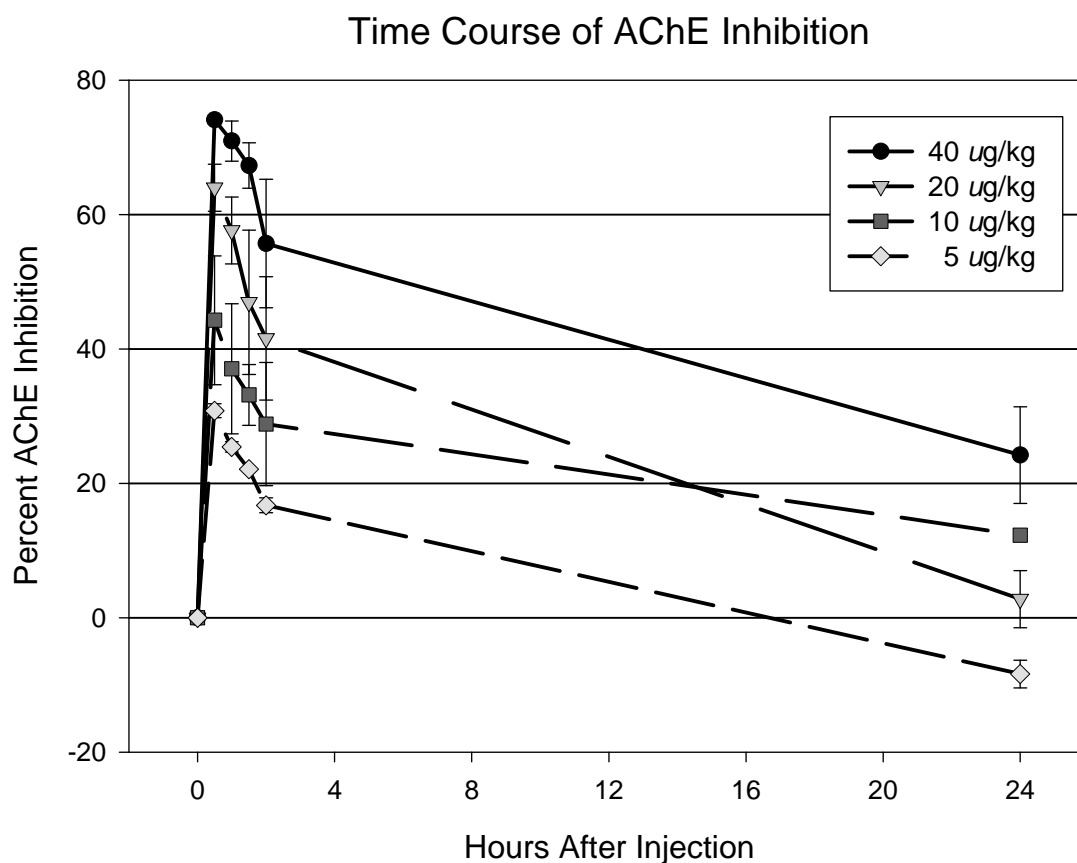

Figure 1. Percent inhibition of AChE over 24 hours following injection. Blood samples were taken at 0 (pre-injection baseline), 0.5, 1, 1.5, 2, and 24 hours following IM injection of four different doses of HUP. Peak AChE inhibition occurred 30 minutes after injection and was dose dependent.
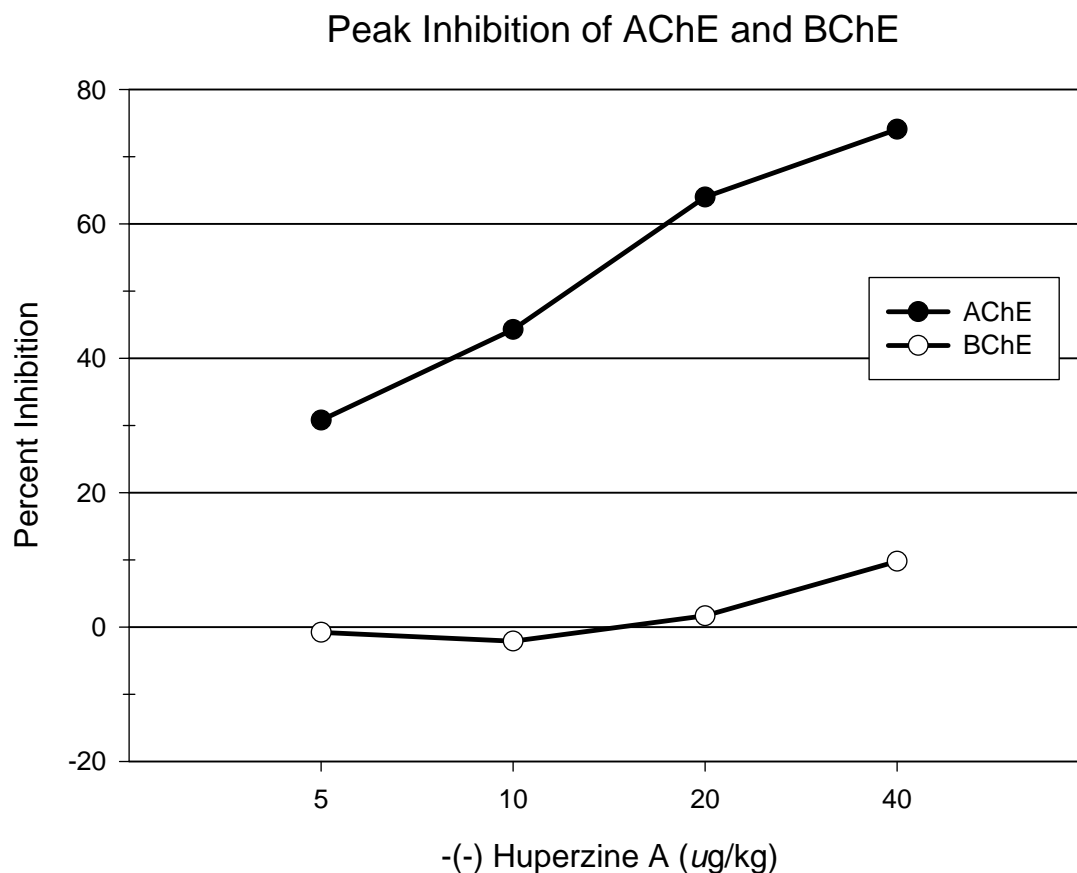
Peak Inhibition of AChE and BChE

Figure 2. Percent inhibition of AChE (filled circles) and BChE (open circles) 30 minutes after IM injection of HUP as a function of dose (plotted on a log scale). For doses ranging from 5 to 40 ug/kg, AChE inhibition ranged from 31 to 74% and BChE inhibition ranged from 0 to 10%.

Behavioral Results

Cognitive-behavioral performance was evaluated using the serial-probe recognition task beginning 30 minutes after injection of each dose: 0 (saline as a vehicle control), 5, 10, 20, or 40 ug/kg. Figure 3 shows results for each dependent measure, accuracy (left panel), trials completed (right panel), and choice reaction time (bottom panel). Compared to the saline vehicle (empty squares), cognitive-behavioral performance following HUP did not differ at any dose. Thus, despite producing greater than 70% inhibition of peripheral AChE at the highest dose, HUP did not alter motivation, attention, and working memory as indexed by the serial-probe recognition task.

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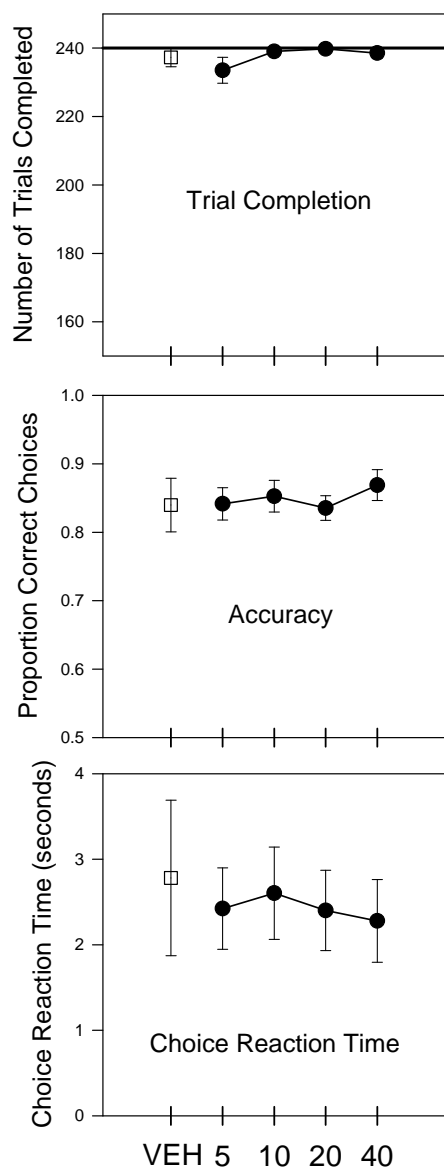
Figure 3. Trials completed, accuracy, and choice reaction time (in seconds) as a function of dose (plotted on a log scale). Empty squares represent performance following saline injection and filled circles represent performance following the injection of HUP at the dose indicated on the x-axis.

DISCUSSION

In rhesus monkeys, we characterized the time-course of peripheral AChE and BChE inhibition following four different doses of HUP that encompassed the therapeutic range of 31 to 74% AChE inhibition. The time of peak AChE inhibition equaled 30 minutes, regardless of dose. BChE inhibition approximated 10% at the highest HUP dose studied (40 ug/kg). Acute dosing produced no performance decrements (or improvements) in trial completion, accuracy, or choice reaction time on the SPR task. Thus, despite inhibiting AChE by as much as 74%, HUP did not produce unwanted side effects. Based on these findings, HUP appears to be behaviorally safe at therapeutic levels.

The present results complement those of previous studies using nonhuman primates. Ye et al. [26] administered HUP to rhesus monkeys that were either aged or pharmacologically challenged with 30 ug/kg scopolamine. Doses of 1 and 10 ug/kg HUP improved choice accuracy on a previously learned delayed spatial memory task in the elderly subjects, and doses of 10 and 100 ug/kg reversed the scopolamine-induced deficits in the younger monkeys. Unfortunately, no data regarding cholinesterase inhibition were reported, so behavior outcomes and doses could not be correlated with AChE inhibition. However, based on AChE inhibition characterized in the present study, doses of 1 to 100 ug/kg would be expected to produce about 8-86% peak inhibition of peripheral AChE. Ye et al. also reported that the beneficial effects of HUP on choice accuracy were often observed at 20 minutes *and* 24 hours (but not 48 hours) after dosing, particularly at the higher doses. This suggests that the time course of inhibition may have been similar to that in the present study, with some AChE inhibition still observed at 24 hours after the 40-ug/kg dose. Another key finding of the study by Ye and colleagues was that delay (retention interval) in the spatial memory task differentially modulated the drug effects on performance. Specifically, scopolamine impaired accuracy proportionally more at the longer delays, and HUP improved accuracy proportionally more at longer delays. An analogous result (differential changes in accuracy as a function of serial position) was not observed in the present study. This suggests that the manipulation of retention intervals over a range of delays (as is common in delayed matching procedures) is a useful means of detecting drug-induced changes in memory functioning. Ou et al. [27], using similar behavioral-pharmacological procedures and young adult rhesus monkeys, extended the findings of Ye et al. to reserpine- and yohimibine-induced memory impairments, finding that 10 ug/kg HUP significantly reversed the drug-induced deficits.

It is worth noting that HUP produced no performance decrement at levels of peripheral AChE inhibition that have produced behavioral disruptions with other acetylcholinesterase inhibitors. For example, Geller et al. [28] examined the delayed match-to-sample performance of baboons following acute soman exposure and measured peripheral AChE inhibition. Exposure to 5 ug/kg soman significantly reduced trial completion and increased response latency, and inhibited AChE by about 60-70%. Lower doses of soman (1-4 ug/kg) did not reliably disrupt performance. Chambers and Chambers [28] exposed rats acutely to paraoxon (with atropine therapy) and produced pronounced behavioral disruptions on a fixed-ratio 10 schedule of food reinforcement. The degree of cortical AChE inhibition at these doses was about 40-60%. Philippens et

al. [30] exposed guinea pigs to acute doses of physostigmine after they acquired shuttlebox avoidance performance and measured peripheral AChE inhibition at 10, 30, and 60 minutes. All three doses of physostigmine significantly reduced avoidance responding, and the effect was clearly dose dependent. For AChE inhibition, mean values ranged from 41-66% and the dose-dependent relation was weak. Mach et al. [31] exposed mice acutely to a physostigmine dose producing about 50% inhibition of peripheral AChE and observed decreased locomotor activity and startle amplitude. Thus, across various species, inhibition of AChE to about 50% of pre-exposure levels can disrupt behavioral functioning. HUP may not produce deficits at comparable levels of AChE inhibition because it is more highly selective for AChE than are the aforementioned cholinesterase inhibitors, thereby leaving other esterases largely unperturbed.

REFERENCES

1. Kluwe WM. Efficacy of pyridostigmine against soman intoxication in a primate model. In:Proceedings of the Sixth Medical Chemical Defense Bioscience Review. Aberdeen Proving Ground, Maryland: US. Army Medical Research Institute of Chemical Defense, 1987; **6**:227-234. AD Bl21516.

2. Dunn MA, Sidell ER. Progress in medical defense against nerve agents. JAMA 1989; **262**:649-652.

3. Kerenyi SZ, Murphy MR, Hartgraves SL. Toxic interactions between repeated soman and chronic pyridostigmine in rodents. Pharmacol Biochem Behav 1990 Oct; **37(2)**:267-271.

4. Dunn MA, Hackley BE Jr, Sidell FR. Pretreatment for nerve agent exposure. In: Sidell FR,Takafuji ET, Franz DR, specialty eds. Medical Aspects of Chemical and Biological Warfare (in series: Zajtchuk R, Bellamy RF, eds. Textbook of Military Medicine). Washington, DC: Office of the Surgeon General at TMM Publications, Department of the Army USA, 1997: 181-196.

5. Ellenhorn MJ (Schonwald S, Ordog G, Wasserberger J, consulting eds.): Ellenhorn's Medical Toxicology: Diagnosis and Treatment of Human Poisoning, Second Edition. Baltimore, Maryland: Williams & Wilkins; 1997: 1286-1287.

6. Marino MT, Schuster BG, Brueckner RP, Lin E, Kaminskis A, Lasseter KC. Population pharmacokinetics and pharmacodynamics of pyridostigmine bromide for prophylaxis against nerve agents in humans. J Clin Pharmacol 1998 Mar; **38(3**):227-235.

7. Wetherell J, Hall T, Passingham S. Physostigmine and hyoscine improves protection against the lethal and incapacitating effects of nerve agent poisoning in the guinea-pig. Neurotoxicology. 2002 Sep;**23(3)**:341-9.

8. von Bredow J, Corcoran K, Maitland G, Kaminskis A, Adams N, Wade J. Efficacy evaluation of physostigmine and anticholinergic adjuncts as a pretreatment for nerve agent intoxication. Fundam Appl Toxicol. 1991 Nov;**17(4)**:782-9.

9. Harris LW, Talbot BG, Lennox WJ, Anderson DR, Solana RP. Physostigmine (alone and together with adjunct) pretreatment against soman, sarin, tabun and VX intoxication. Drug Chem Toxicol. 1991;**14(3)**:265-81.

10. Anderson DR, Harris LW, Lennox WJ, Solana RP. Effects of subacute pretreatment with carbamate together with acute adjunct pretreatment against nerve agent exposure. Drug Chem Toxicol. 1991;**14(1-2)**:1-19.

11. Solana RP, Gennings C, Carter WH Jr, Anderson D, Lennox WJ, Carchman RA, Harris LW. Evaluation of the efficacy of two carbamates, physostigmine and pyridostigmine, when used in conjunction for protection against organophosphate exposure. Fundam Appl Toxicol. 1990 Nov;**15(4)**:814-9.

12. Grunwald J, Raveh L, Doctor BP, Ashani Y. Huperzine A as a pretreatment candidate drug against nerve agent toxicity. Life Sci.1994;**54(14)**:991-7.

13. Lallement G, Demoncheaux JP, Foquin A, Baubichon D, Galonnier M, Clarencon D, Dorandeu F. Subchronic administration of pyridostigmine or huperzine to primates: compared efficacy against soman toxicity. Drug Chem Toxicol. 2002 Aug;**25(3)**:309-20.

14. Lallement G, Baille V, Baubichon D, Carpentier P, Collombet JM, Filliat P, Foquin A, Four E, Masqueliez C, Testylier G, Tonduli L, Dorandeu F. Review of the value of huperzine as pretreatment of organophosphate poisoning. Neurotoxicology. 2002 May;**23(1)**:1-5.

15. Lallement G, Foquin A, Dorandeu F, Baubichon D, Carpentier P. Subchronic administration of various pretreatments of nerve agent poisoning. II. Compared efficacy against soman toxicity. Drug Chem Toxicol. 2001 May;**24(2)**:165-80.

16. Clark MG, Sun W, Myers TM, Bansal R, Doctor BP, Saxena A. Effects of physostigmine and human butyrylcholinesterase on acoustic startle reflex and prepulse inhibition in C57BL/6J mice. Pharmacol Biochem Behav. 2005 Jul;**81(3)**:497-505.

17. Liu WF. Effects of cholinesterase inhibitors on a two-component chained schedule performance in rats. Neurotoxicol Teratol. 2000 May-Jun;**22(3)**:389-96.

18. Bizot JC. Effects of various drugs including organophosphorus compounds (OPC) and therapeutic compounds against OPC on DRL responding. Pharmacol Biochem Behav. 1998 Apr;**59(4)**:1069-80.

19. Philippens IH, Wolthuis OL, Busker RW, Langenberg JP, Melchers BP. Side effects of physostigmine as a pretreatment in guinea pigs. Pharmacol Biochem Behav. 1996 Sep;**55(1)**:99-105.

20. Frederick DL, Schulze GE, Gillam MP, Paule MG. Acute effects of physostigmine on complex operant behavior in rhesus monkeys. Pharmacol Biochem Behav. 1995 Apr;**50(4)**:641-8.

21. Preston KL, Schuster CR, Seiden LS. Methamphetamine, physostigmine, atropine and mecamylamine: effects on force lever performance. Pharmacol Biochem Behav. 1985 Nov;**23(5)**:781-8.

22. Zangara A. The psychopharmacology of huperzine A: an alkaloid with cognitive enhancing and neuroprotective properties of interest in the treatment of Alzheimer's disease. Pharmacol Biochem Behav. 2003 Jun;**75(3)**:675-86.

23. Diamond B, Johnson S, Torsney K, Morodan J, Prokop B, Davidek D, Kramer P. Complementary and alternative medicines in the treatment of dementia: an evidence-based review. Drugs Aging. 2003;**20(13)**:981-98.

24. Gordon RK, Haigh JR, Garcia GE, Feaster SR, Riel MA, Lenz DE, Aisen PS, Doctor BP. Oral administration of pyridostigmine bromide and huperzine A protects human whole blood cholinesterases from ex vivo exposure to soman. Chem Biol Interact. 2005 Dec 15;**157-158**:239-46. Epub 2005 Oct 26.

25. Myers TM, Clark MG. Serial-probe recognition in rhesus macaques: effects of midazolam. Pharmacol Biochem Behav. 2006 Nov;**85(3)**:555-61. Epub 2006 Dec 1.

26. Ye JW, Cai JX, Wang LM, Tang XC. Improving effects of huperzine A on spatial working memory in aged monkeys and young adult monkeys with experimental cognitive impairment. J Pharmacol Exp Ther. 1999 Feb;**288(2)**:814-9.

27. Ou LY, Tang XC, Cai JX. Effect of huperzine A on working memory in reserpine- or yohimbine-treated monkeys. Eur J Pharmacol. 2001 Dec 21;**433(2-3)**:151-6.

28. Geller I, Hartmann RJ, Leal BZ, Haines RJ, Gause EM. Effects of the irreversible acetylcholinesterase inhibitor soman on match-to-sample behavior of the juvenile baboon. Proc West Pharmacol Soc. 1984;**27**:217-21.

29. Chambers JE, Chambers HW. Short-term effects of paraoxon and atropine on schedule-controlled behavior in rats. Neurotoxicol Teratol. 1989 Sep-Oct;**11(5)**:427-32.

30. Philippens IH, Melchers BP, Wolthuis OL. Active avoidance behavior in guinea pigs: effects of physostigmine and scopolamine. Pharmacol Biochem Behav. 1992 Jun;**42(2)**:285-9.

31. Mach M, Grubbs RD, Price WA, Paton SJ, Lucot JB. Behavioral changes after acetylcholinesterase inhibition with physostigmine in mice. Pharmacol Biochem Behav. 2004 Nov;**79(3)**:533-40.